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(54) Title: A METHOD TO INCREASE THE EXCRETION OF NON-STEROL ENDOGENOUS HYDROPHOBIC
SUBSTANCES BY INCREASING EXCRETION OF FAT VIA THE FAECES

(57) Abstract: The subject invention concerns a method to increase the excretion of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof by increasing excretion via the faeces of the hydrophobic substance or metabolic derivative thereof characterised in that the excretion of fat via the faeces is increased. The purpose of the invention is to prevent or treat conditions associated with the accumulation of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof, such substances being unconjugated bilirubin and protoporphyrin. More particularly, lipstatin, orlistat, tetrahydrolipstatin, polyol fatty acids like olestra, and non-conventional doses of dietary jet are used to treat neonatal jaundice, haemolytic or erythropoietic protoporphyria.

A method to increase the excretion of non-sterol endogenous hydrophobic substances by increasing excretion of fat via the faeces

Summary of the invention

5 The invention concerns a method to increase the excretion of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof by increasing excretion via the faeces of the hydrophobic substance or metabolic derivative thereof. Also the invention concerns a method for prevention or treatment of conditions associated with the accumulation of non-sterol endogenous hydrophobic substances or
10 metabolic derivatives thereof.

Background of the invention

 Disturbance of the homeostasis of non-sterol endogenous hydrophobic compounds in mammals, specifically in humans, can lead to accumulation of
15 detrimental amounts of these compounds.

 One example of such a compound is bilirubin. Under physiological conditions, bilirubin undergoes two conjugation reactions with glucuronic acid, derived from UDP-glucuronide, which results in the formation of bilirubin diglucuronide. Bilirubin diglucuronide is significantly more water-soluble than the parent compound,
20 unconjugated bilirubin (UCB), and can be readily excreted via the bile into the faeces. The two conjugation reactions are catalysed by the hepatic enzyme uridine diphosphoglucuronosyl transferase (h-UDPGTbil, EC 2.4.1.17). In Crigler Najjar's disease (CN) the activity of h-UDPGTbil is completely absent (CN type I) or significantly reduced (CN type II), leading to increased serum concentrations of UCB.
25 Increased serum levels of UCB are also found during the neonatal period, especially in preterms, during increased rates of haemoglobin degradation (for example sickle cell crisis, anaemic crisis in G6PD-deficient individual, ABO-antagonism or other forms of immune or non-immune hemolysis), or during impaired hepatic conjugation efficiency (for example viral infections, metabolic diseases, and others)(for review, see
30 Chowdhury et al., Hereditary jaundice and disorders of bilirubin metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Basis of Inherited Disease. New York: McGraw-Hill, Inc. 1995:2161-2208).

High serum concentrations of UCB are associated with accumulation in other organs of the body, among which the central nervous system, and with toxic effects on the central nervous system. In order to keep serum concentrations of UCB below 250 $\mu\text{mol/L}$, unconjugated hyperbilirubinemia is conventionally treated with phototherapy for many hours daily. Phototherapy (wavelength 400-460 nm) results in the formation of a variety of photoproducts which can be secreted into the bile, however, only at a relatively slow rate. After biliary secretion, the configurational isomers can spontaneously revert to the normal configuration and be absorbed from the intestinal lumen. These features of the configurational isomers obviously decrease the efficacy of phototherapy. Whereas Crigler-Najjar's disease patients (in particular type I) are usually treated lifelong by phototherapy at home, neonates with unconjugated hyperbilirubinemia are generally admitted to hospitals for phototherapeutic treatment. If phototherapy fails to lead to clinically acceptable serum concentrations of UCB, patients may need to undergo one or more exchange transfusions, which comprises a high-risk therapy with considerable morbidity and even mortality.

Alternative strategies for the treatment of unconjugated hyperbilirubinemia involve the capture of UCB or of its photoisomers in the intestinal lumen, thereby preventing their intestinal uptake and enterohepatic circulation. The first results of the intestinal UCB capture approach date back to 1983. It was demonstrated that the enteral administration of agar could serve as an adjunct to phototherapy in neonates with unconjugated hyperbilirubinemia (Odell et al., *Pediatr Res* 1983;17:810-814). Also, the oral administration of activated charcoal to Gunn rats was associated with a decrease in serum bilirubin concentration (Davis et al., *Pediatr Res* 1983;17:208-209). The capture of bilirubin in the intestinal lumen has also been attempted with cholestyramine, but only a modest benefit was obtained (Nicolopoulos et al., *J Pediatr* 1978;93:684-688, Tan et al., *J Pediatr* 1984;104:284-286). Nagyvary described and patented the use of chitosan (a polymer of N-acetyl-D-glucosamine units) to treat hyperbilirubinemia, based on the intraluminal binding of bilirubin in the intestine (US 4,363,801). Disadvantage of the use of these hydrophilic resins or resin-like materials is that these will bind a great variety of other useful components, which subsequently will be excreted. Other patents on the application of UCB adsorption to treat hyperbilirubinemic states include US 5,200,181, in which a bilirubin converting enzyme is used; US 4,593,073, in which amino acid containing polymers are used, US

5,804,218 in which zinc salts are used. None of these alternative strategies has resulted in a practically used therapy.

Another example of a non-sterol endogenous hydrophobic compound in mammals, whose accumulation can lead to detrimental consequences, is protoporphyrin. Protoporphyrin (PP) is a hydrophobic intermediate in the biosynthesis of heme. Heme is an iron-containing, prosthetic group in many proteins, which function in for example oxygen en electron transport, H_2O_2 generation and degeneration, and nitric oxide synthesis. Catalysed by the enzyme ferrochelatase (EC 4.99.1.1), PP is converted into heme through the addition of a Fe^{2+} -atom. Although all mammalian cells synthesise heme, the major site is the bone marrow, where approximately 85% of the body's heme is produced for the formation of hemoglobin. The second major site of heme synthesis in mammals is the liver (see for review Kappas et al., *The Porphyrrias*. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Basis of Inherited Disease*. New York: McGraw-Hill, Inc. 1995:2103-2159).

Under several pathophysiological conditions, for example in the disease erythropoietic protoporphyria (EPP), PP accumulates in body. EPP is an autosomal dominant, inherited disease, which is characterised by a strongly reduced activity of the ferrochelatase enzyme. Under the pathophysiological condition of PP accumulation, particularly in erythrocytes, liver and faeces increased PP concentrations are found (Romslo et al., *Arch Dermatol* 1982;118:668-671, Beukeveld et al., *Clin Chem* 1987;33:2164-2170). The clinical consequences of increased concentrations of PP in the body can be exemplified by the symptoms of EPP. At young age, EPP patients have a cutaneous photosensitivity in light-exposed areas. The mechanism of the photosensitivity involves the generation of free oxygen radicals from accumulated PP in the skin, under influence of light (wavelength 400-410 nm). The reactive oxygen radicals damage primarily the mitochondria and cellular membranes, leading to severely discomforting skin lesions (burn-like lesions, itching, oedema, scarring).

The disposal from the body of PP involves biliary secretion and subsequent loss via the stools. It is not known whether the highly hydrophobic parent molecule PP can be reabsorbed by the intestinal mucosa and undergoes enterohepatic cycling. Strong, indirect support for this possibility can be derived from the observed beneficial effects of cholestyramine on PP accumulation in EPP. It appeared that the administration of cholestyramine, in analogy to its use in hyperbilirubinemia, improved photosensitivity

and reduced hepatic PP content (Tishler et al., Methods Find Exp Clin Pharmacol 1985;7:485-491, McCullough et al., Gastroenterology 1988;94:177-181).

Description of the invention

5 The present invention is directed at a novel mechanism of intestinal capture of non-sterol endogenous hydrophobic compounds, specifically the induction of increased fat excretion via the faeces (steatorrhoea).

10 The capture of non-sterol endogenous hydrophobic compounds by luminal fat differs from the previous approaches. Calcium phosphate and activated charcoal are suggested to bind UCB for instance or its photoisomers by an adsorption process. Cholestyramine, applied in conditions in which UCB, PP and plant sterols were increased, is applied as a resin, which binds the respective molecules. The same is true for the fibrous material chitosan.

15 By increasing the lipophilic phase, or amount of fat, in the intestinal lumen, as disclosed according to the invention, hydrophobic compounds will dissolve or diffuse into the generated apolar phase. The apolar, lipophilic phase persists throughout the digestive tract and will drag hydrophobic compounds along the intestinal tract, which eventually will be excreted. The hydrophobic phase is virtually impermeable for polar detergents, such as bile salts. It can be expected that the absorption of fat-soluble
20 compounds such as fat soluble vitamins, such as vitamin A, vitamin D, vitamin E, and vitamin K, will also be inhibited. To compensate for this, an increased dietary intake, either in natural or in water-soluble form, may be warranted. The induction of increased faecal fat excretion by any means increases the disposal of endogenous hydrophobic substances such as UCB and PP from the body, at least under conditions of their
25 previous accumulation. Thus the present invention provides a method to prevent or treat conditions such as neonatal jaundice, haemolytic jaundice and erythropoietic protoporphyria.

30 In the examples it is described that in Gunn rats the induction of fat malabsorption, and thus, of increased faecal fat excretion, was dose-dependently related to decreased plasma UCB concentrations. A strong, inverse correlation was observed between the amount of faeces produced and the plasma UCB concentration in the rats studied. Based on the nature of the effect, namely hydrophobic diffusion, it can be anticipated that the same strategy would allow increasing the disposal from the body of

other hydrophobic compounds upon their accumulation under pathophysiological circumstances.

Admittedly, parts of the presently proposed concept have been appreciated quite some time previously when addressing other problems. In particular is referred to strategies to decrease plasma levels of cholesterol. The plasma cholesterol concentration is related to the intestinal absorption of cholesterol, derived either from the diet or from the bile. As exemplified by studies employing sucrose fatty acid polyesters, decreasing the intestinal (re)absorption of cholesterol is associated with an improved plasma lipid and lipoprotein profile (Jandacek, Int J Obes 1984;8 Suppl:13-21, Jandacek et al., Metabolism 1990;39:848-852, US 3,954,976, US 4,005,195, US 4,005,196). The specific use of sucrose polyesters to detoxify humans and lower animals after accidental or chronic ingestion of lipophilic toxins such as insecticides (for example, DDT, Kepone), herbicides (for example, PCP) or industrial chemicals (for example, polychlorinated biphenyls (PCB's), polybrominated biphenols (PBB's)) has also been patented (US 4,241,054).

The present invention however is directed at the general approach to increase the disposal from the body of non-sterol endogenous hydrophobic compounds, such as UCB and PP, namely by inducing/increasing the faecal excretion of a hydrophobic (lipophilic) phase, through whatever mechanism. The previous disclosures are directed at elimination of a different category of compounds to the invention, using different means. The previous disclosures have been available in the prior art for a long time and nevertheless the skilled person in the field of for instance hyperbilirubinemia or protoporphyria have been occupied with totally different concepts of treatment.

Several strategies to induce increased faecal fat excretion can be envisaged for carrying out the method according to the invention. The various physiological processes involved in fat absorption can be influenced for example in the following ways:

- inhibition of lipolysis: Dietary fat consists for 92-96% of triacylglycerols, which have to be hydrolysed by lipolytic enzymes before they can be absorbed. Interference with the lipolysis can be obtained by inhibition of the lipase enzymes, operational in the gut lumen, for example by orlistat (Hochuli et al., J Antibiot (Tokyo) 1987;40:1086-1091, Weibel et al., J Antibiot (Tokyo) 1987;40:1081-1085) or functionally and/or structurally related compounds (Yoshinari et al., J Antibiot (Tokyo) 1994;47:1376-

1384) (see also for example US. 4,598, 089, EP 185 359 and EP 444 482). Inhibition of lipolysis could also be achieved by using compounds from the oxetanone group (US 4,931,463) or the esterastin group (US 4,202,824). The validity of the concept to induce fat malabsorption by this principle has been described in the prior art (Hogan et al., Int J Obes 1987;11 Suppl 3:35-42, Fernandez and Borgstrom, Biochim Biophys Acta 1989;1001:249-255 and Biochim Biophys Acta 1989;1001:249-255, Hauptman et al., Am J Clin Nutr 1992;55:309S-313S, Reitsma et al., Metabolism 1994;43:293-298, Isler et al., Br J Nutr 1995;73:851-862).

- inactivation of fatty acid and monoacylglycerol solubilisation: Long-chain fatty acids and monoacylglycerols, the metabolic products of intraluminal lipolysis, are hardly soluble in the aqueous environment of the small intestine. Under physiological circumstances, bile components (bile salts, phospholipids) increase their aqueous solubility (a process also known as solubilisation), by the formation of complex aggregates (micelles, vesicles), consisting of fatty acids, monoacylglycerols, bile salts, phospholipids, cholesterol. Interference with this process of solubilisation, for example by inhibiting the enteral influx of bile or by decreasing the soluble ("active") concentration of bile salts, has been demonstrated to be associated with faecal fat excretion and, thus, to impair intestinal fat absorption (Poley et al., Gastroenterology 1976;71:38-44, Graham and Sackman, Gastroenterology 1982;83:638-644, DeVizia et al., Pediatr Res 1985;19:800-806, Chappell et al., J Pediatr 1986;108:439-447, Potter et al., Nutrition 1990;6:309-312, Sandberg et al., Am J Clin Nutr 1994;60:751-756, Carnielli et al., Am J Clin Nutr 1995;61:1037-1042, Mabayo et al., Lipids 1995;30:839-845, Xu C et al., J Dairy Sci 1998;81:2173-7). Increasing the viscosity of the luminal phase, such as by carboxymethylcellulose, will also impair fat absorption and thus increase faecal fat excretion (Smits et al., Poult Sci 1998;77:1534-9). Relevant to this approach is the described use of such products in elimination of laxative effects of low calorie fat materials (EP 236288).

- inhibition of lipid translocation across the apical membrane of the intestinal mucosal cells: Carrier-mediated uptake mechanisms for lipids have been hypothesised and partially identified at the level of the brush border membrane of the small intestinal mucosa (Compassi et al., Biochemistry 1995;34:16473-16482, Schoeller et al., Clin Invest Med 1995;18:380-388, Fitscher et al., Proc Soc Exp Biol Med 1996;212:15-23, Schulthess et al., J Lipid Res 1996;37:2405-2419, Boffelli et al., Biochemistry

1997;36:10784-10792). Candidate proteins involved in these processes have been indicated and it is reasonable to assume that specific inhibitors of this (these) carrier system(s) would inhibit the absorption of luminal lipids, leading to increased faecal fat excretion. An example of this phenomenon can be derived from the studies by
5 Stremmel et al. (J Clin Invest 1985;75:1068-1076), in which the translocation of a fatty acid across brush border membrane vesicles could be inhibited by the incubation with a specific antibody.

- inhibition of an intracellular event of fat absorption: fatty acid/monoacylglycerol reacylation, chylomicron assembly, and/or basolateral chylomicron secretion: After
10 lipids have entered the small intestinal mucosal cell, they are reassembled into chylomicrons, either with (monoacylglycerols, fatty acids, lysophospholipids, unesterified sterols) or without (fraction of unesterified sterols, phospholipids) prior to reacylation. The intracellular events in fat absorption and chylomicron assembly are only partially understood (see for review Tso P. Intestinal lipid absorption. In: Johnson
15 LR, ed. Physiology of the gastrointestinal tract. New York: Raven Press, 1994:1867-1907). One of the factors which recently has been identified to be of crucial importance for proper chylomicron assembly and secretion is the Microsomal Triglyceride Transfer Protein (Wetterau et al., Science 1992;258:999-1001, Wetterau et al., Biochim Biophys Acta 1997;1345:136-150). Inhibition of this protein has been demonstrated to induce
20 net fat malabsorption, by means of impaired assembly and secretion of chylomicrons, and the subsequent shredding of the lipid-loaded mucosal cell into the intestinal lumen.

---This form of strategy, in which an intracellular event involved in fat absorption is inhibited, would also be applicable to induce an increased faecal fat excretion with the aim to increase disposal of unconjugated bilirubin or protoporphyrin. The use of MTP
25 inhibitors to remove plant sterols from the body has for instance been described in WO 98 31225.

The purpose to increase faecal fat excretion cannot only be accomplished by interference with the physiological processes involved in fat absorption. Other approaches are the administration of non-absorbable hydrophobic compounds or the
30 administration of hydrophobic compounds in a non-absorbable amount.

Non-absorbable hydrophobic compounds include for example (poly)esters of fatty acids and sugars or alcohols. Administration of olestra, one particular type of sucrose polyester, has been demonstrated to decrease intestinal absorption and decrease

body disposal of the hydrophobic sterol cholesterol in hypercholesterolemic man (Mellies et al., Am J Clin Nutr 1983;37:339-346, Jandacek et al., Metabolism 1990;39:848-852) or of hydrophobic environmental pollutants in gerbils (Jandacek et al., Drug Metab Rev 1982;13:695-714, Mutter et al., Toxicol Appl Pharmacol 5 1988;92:428-435). The specific use of sucrose polyesters to detoxify humans and lower animals after accidental or chronic ingestion of toxic lipophilic materials has also been patented (US 4,241,054).

An alternative method to obtain an increased faecal excretion of fat involves the administration of supraphysiological amounts of conventional dietary fats (Fomon et 10 al., Am J Clin Nutr 1970;23:1299-1313) which cannot be absorbed quantitatively. Endogenous hydrophobic compounds dissolve in dietary fats and are concomitantly excreted.

The present invention is also directed at pharmaceutical compositions comprising the compound to be administered as active compound in an effective amount according 15 to any of the abovementioned strategies for prevention or treatment of conditions associated with the accumulation of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof together with a pharmaceutically acceptable carrier. According to the invention the pharmaceutical compositions are directed at the prevention or treatment of conditions that are associated with the accumulation of 20 unconjugated bilirubin or protoporphyrin, such as neonatal jaundice, haemolytic jaundice or erythropoietic protoporphyria.

The present invention is also directed at the use of any compound being the compound to be administered as active compound according to any of the abovementioned strategies for the manufacture of a pharmaceutical composition for 25 prevention or treatment of conditions associated with the accumulation of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof, such as the accumulation of unconjugated bilirubin or protoporphyrin, possibly leading to, for example, neonatal jaundice, haemolytic jaundice or erythropoietic protoporphyria.

The active compound according to the invention has to be delivered in the 30 intestinal lumen in a therapeutically effective amount. The form of administration of the active compound or pharmaceutical composition containing the active compound according to the invention can be any suitable form known to one of ordinary skill in the art for administering a therapeutically active agent to the intestinal lumen, e.g. oral,

enteral or rectal administration. Selection of the dosage is to be made by anyone of ordinary skill in the art of medicine, giving consideration to age, sex, size, fat mass/lean body mass ratio and the condition the recipient is suffering or to be prevented from suffering.

- 5 The following examples illustrate the feasibility of the subject invention and are not intended to limit the scope of the invention. The references cited are incorporated by citation in the subject description.

Examples

10

Example 1

- Male Gunn rats (body weight 300-350 g) were fed a high-fat (16 wt%) chow diet for 2 weeks. Major long-chain fatty acid composition of the diet: palmitic acid, 31.9%; stearic acid, 5.2%; oleic acid, 32.7%; linoleic acid 30.2%; Hope Farms, Woerden, The Netherlands). After two weeks, animals were divided in three groups (each n=5). Each group received for another 6 days grounded and dried high fat diet, supplemented without (control) or with the lipase inhibitor orlistat (Xenical®, 200 mg/kg; 800 mg/kg). Blood samples were collected obtained in EDTA-containing cups by tail bleeding at day 6, and plasma was obtained by centrifugation (10 min, 2000 rp, 4 °C) and stored under light-protected conditions until analysis (within hours after sampling). Bilirubin concentration in plasma was determined by automated analysis, based on the diazo method (Novros et al., Clin Chim Acta 25:1891-9; 1979). The results are depicted in Fig. 1. A significant decrease of the plasma concentration of unconjugated bilirubin as a result of an orlistat containing diet was observed.

25

Example 2

- Male Gunn rats (body weight 300-350 g) were fed a high-fat (16 wt%) chow diet for 2 weeks. Major long-chain fatty acid composition of the diet: palmitic acid, 31.9%; stearic acid, 5.2%; oleic acid, 32.7%; linoleic acid 30.2%; Hope Farms, Woerden, The Netherlands). After two weeks, animals were divided in three groups (each n=5). Each group received for another 6 days grounded and dried high fat diet, supplemented without (control) or with the lipase inhibit or orlistat (Xenical®, 200 mg/kg; 800 mg/kg). From day 4 till day 6, faeces was quantitatively collected and weighed. Faeces

30

production was compared with plasma bilirubin concentrations at day 6 (see Fig. 1) in individual animals. The results are represented in Fig.2. The correlation line was characterised by the equation: $Y = 293.6 - 9.9.X$; in which Y = unconjugated bilirubin concentration in plasma, and X = total faeces production, $R = -0.67$, $P = 0.006$. It was found that the plasma concentration of unconjugated bilirubin was inversely related to the amount of faeces production as a result of an orlistat containing diet.

Description of the figures

Fig.1: The effect of dietary supplementation with orlistat for 6 days on plasma concentration of unconjugated bilirubin in male Gunn rats (each group n=5).

Fig.2: Inverse relation of the plasma concentration of unconjugated bilirubin and the amount of faeces production in Gunn rats on orlistat-containing diets.

Claims

1. Method to increase the excretion of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof by increasing excretion via the faeces of the hydrophobic substance or metabolic derivative thereof characterised in that the excretion of fat via the faeces is increased.
2. Method according to claim 1, for prevention or treatment of conditions associated with the accumulation of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof.
3. Method according to claim 1 or 2, characterised in that the non-sterol endogenous hydrophobic substances are unconjugated bilirubin or protoporphyrin.
4. Method according to claim 2 or 3, characterised in that the conditions to be prevented or treated are associated with the accumulation of unconjugated bilirubin or protoporphyrin, such as neonatal jaundice, haemolytic jaundice or erythropoietic protoporphyria.
5. Method according to any of claims 1-4, comprising administering a compound that is an inhibitor of lipolysis such as oxetanone derivatives, esterastin derivatives, lipstatin or orlistat, tetrahydrolipstatin and functionally and/or structurally related compounds
6. Method according to any of claims 1-4, comprising administering a compound that inactivates fatty acid and monoacylglycerol solubilisation.
7. Method according to any of claims 1-4, comprising administering a compound that is an inhibitor of the translocation of fatty acids from the intestinal lumen across the apical membrane of the intestinal mucosal cells.
8. Method according to any of claims 1-4, comprising administering a compound that is an inhibitor of any intracellular event of fat absorption, such as fatty acid and/or monoacylglycerol reacylation and/or the assembly and/or secretion of chylomicrons from the intestinal mucosal cells, such compounds for example being MTP inhibitors.
9. Method according to any of claims 1-4, comprising administering a compound that is a non-digestible hydrophobic compound such as a polyol fatty acid ester, such a compound for example being a sucrose fatty acid ester with at least 4 fatty acid esters or for example olestra.
10. Method according to any of claims 1-4, comprising administering a non-conventional dosage of dietary fat or dietary fat containing food products as supplement to a conventional diet.

11. Pharmaceutical composition comprising the compound to be administered according to any of claims 5-10 as active compound in an effective amount for prevention or treatment of conditions associated with the accumulation of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof together with a
5 pharmaceutically acceptable carrier.

12. Pharmaceutical composition according to claim 11 in which the conditions to be prevented or treated are associated with the accumulation of unconjugated bilirubin or protoporphyrin, such as neonatal jaundice, haemolytic jaundice or erythropoietic protoporphyria.

10 13. Pharmaceutical composition according to claim 11 or 12 supplemented with additives that readily dissolve in fat, such as fat soluble vitamins, such as vitamin E, vitamin A, vitamin D and vitamin K.

14. Use of any compound being the compound to be administered according to any of claims 5-10 as active compound for manufacture of a pharmaceutical composition for
15 prevention or treatment of conditions associated with the accumulation of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof.

15. Use of any compound according to claim 14 in which the conditions to be prevented or treated are associated with the accumulation of unconjugated bilirubin or protoporphyrin, such as neonatal jaundice, haemolytic jaundice or erythropoietic
20 protoporphyria.

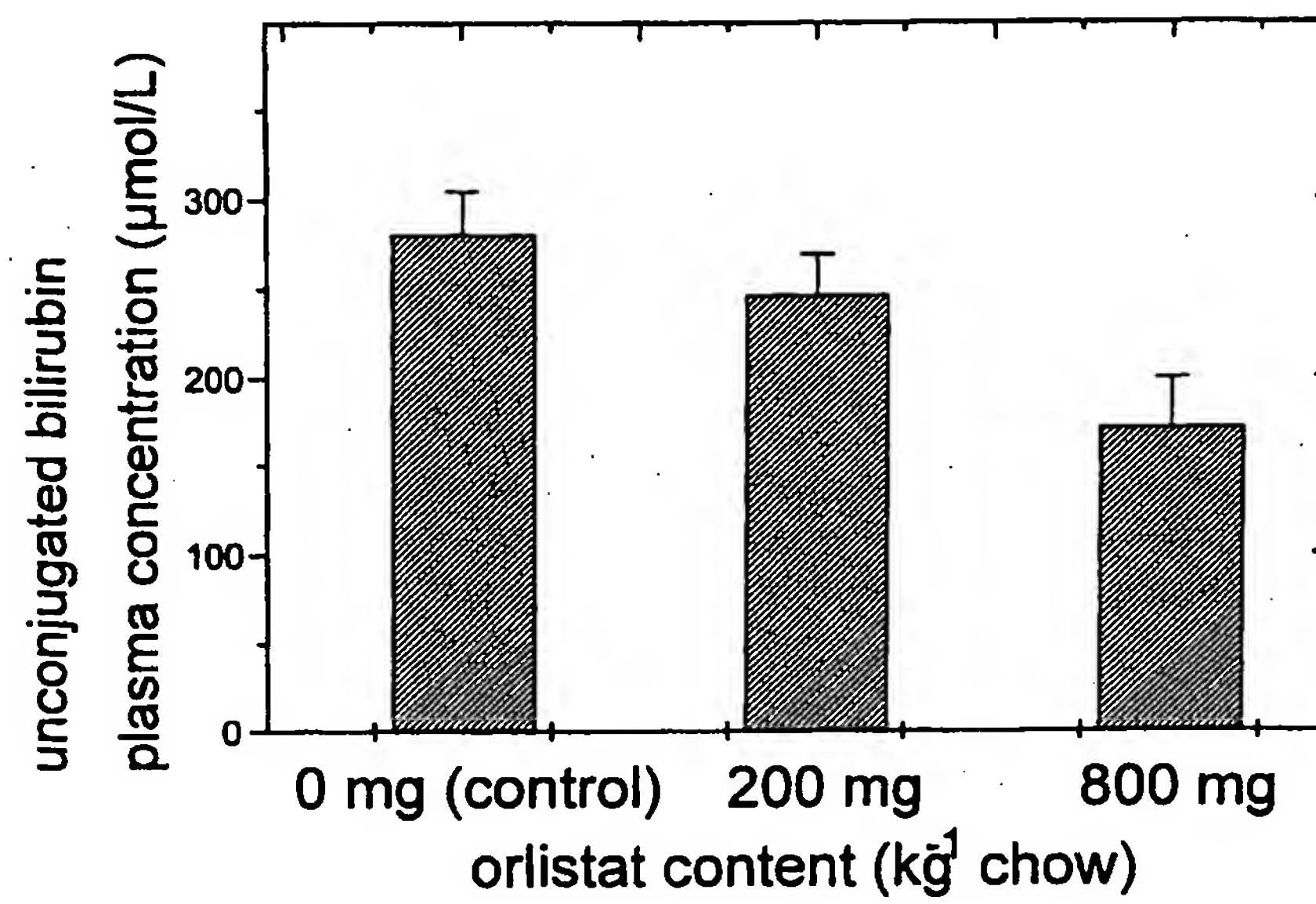
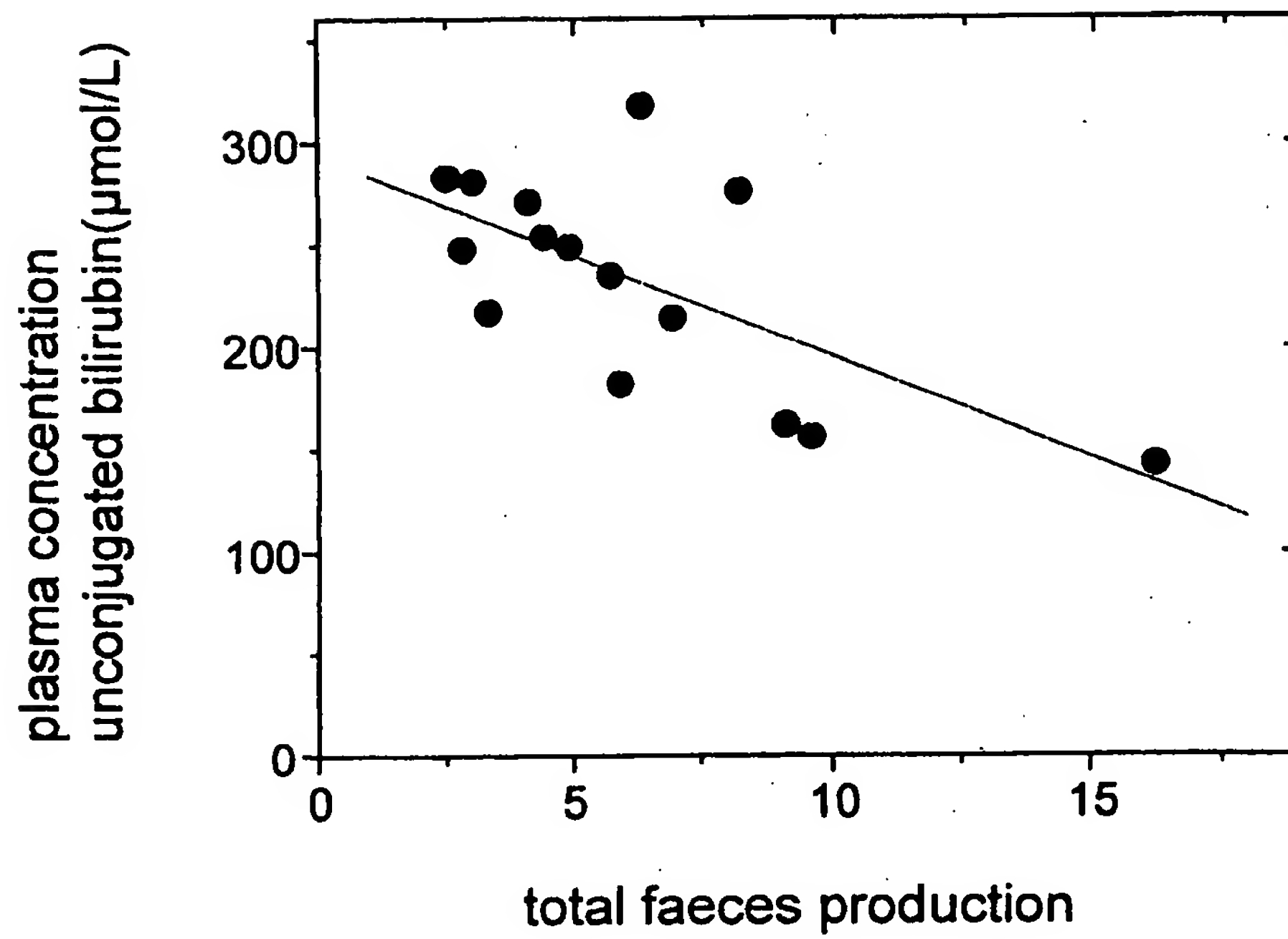
Fig. 1

Fig. 2

INTERNATIONAL SEARCH REPORT

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PCT/NL 99/00492

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched.

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 89 06137 A (MASSACHUSETTS INST TECHNOLOGY) 13 July 1989 (1989-07-13) abstract page 3, line 10 -page 5, line 33; claims 1-12; example 1 ---	1-4
X	US 4 363 801 A (NAGYVARY JOSEPH J) 14 December 1982 (1982-12-14) column 2, line 32 -column 3, line 7; claims 1-7 ---	1-4, 6-8, 11, 12, 14, 15
X	US 5 665 775 A (SHUQAIR MA AN M ET AL) 9 September 1997 (1997-09-09) abstract column 2, line 11 -column 3, line 33; claims 1-32; example 7 --- -/-	1-4, 6-15

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 199 888 A (UNIV ROCKEFELLER) 5 November 1986 (1986-11-05) abstract ---	1-4
X	SCHWIZER, W. ET AL: "Role of lipase in the regulation of upper gastrointestinal function in humans" AM. J. PHYSIOL. (1997), 273(3, PT. 1), G612-G620 , XP000865896 abstract ---	1-5, 11, 12
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/NL 99/ 00492

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-10
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-10
are directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☒ Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/NL 99 00492

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-15 relate to a compound/product/method/apparatus defined (inter alia)

by reference to the following parameters:

P1: Compound that increases the excretion of non-sterol endogenous hydrophobic substances or metabolic derivatives thereof.

P2: Compound that inactivates fatty acid and monoacylglycerol solubilisation.

P3: Compound that is an inhibitor of the translocation of fatty acids from the intestinal lumen across the apical membrane of the intestinal mucosa cells.

P4: Compound that is an inhibitor of any intracellular event of fat absorption, such as fatty acid and/or monoacylglycerol reacylation and/or the assembly and/or secretion of chylomicrons from the intestinal mucosal cells, such compound for example being microsomal triglyceride transfer protein (MTP) inhibitors.

The use of these parameters in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is impossible to compare the parameters the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to the compounds specifically mentioned in the claims and in examples 1-2.

Regarding claim 5, the terms "functionally and/or structurally related compounds" and "esterastin derivatives" are not clear.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

ional Application No

PCT/NL 99/00492

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